

The University of Chicago

EFFECT OF LIGHT
AND TEMPERATURE ON FLORAL
INITIATION IN COCKLEBUR
AND BILOXI SOYBEAN

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY
1940

By
WILLIAM E. SNYDER

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE, Vol. 102, No. 2, 1940



The University of Chicago

EFFECT OF LIGHT
AND TEMPERATURE ON FLORAL
INITIATION IN COCKLEBUR
AND BILOXI SOYBEAN

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1940

By
WILLIAM E. SNYDER

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE, Vol. 102, No. 2, 1940

EFFECT OF LIGHT AND TEMPERATURE ON FLORAL INITIATION IN COCKLEBUR AND BILOXI SOYBEAN¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 516

WILLIAM E. SNYDER

(WITH FIVE FIGURES)

Introduction

In recent work with Biloxi soybean (*Soja max*) and cocklebur (*Xanthium pennsylvanicum*), HAMNER (6) has shown that photoperiodic induction takes place as a result of reactions which occur during both the light and the dark period of a photoinductive cycle. Disregarding the results dealing with light intensity and temperature, the following scheme may illustrate the response on the part of the plant to given treatments. For purposes of simplicity, the only variations introduced are those of duration of the photoperiods and the dark periods during particular treatments with cyclic alternations of light and darkness.

1. If the light period is of a given duration (about 12 hours) and the dark period is also of a given duration, the plants will flower as a result of treatment.
2. If the dark period is shorter than 8 hours, the plants will fail to flower, regardless of the duration of the photoperiod.
3. If the photoperiod is very short, even though the dark period is relatively long, the plants may fail to flower; the response depends upon the previous conditions to which the plant has been exposed.
4. If both the photoperiods and the dark periods are relatively short, the plants will fail to flower, and such treatment serves to inhibit flowering following subsequent photoperiodic cycles which might otherwise prove effective in bringing about induction.

As a result of these observations, HAMNER has presented the following relationship with regard to Biloxi soybean and cocklebur. If A represents the changes or conditions related to exposure to light, and B represents those related to exposure to darkness, then A, B→C represents the interrelationship between A and B which brings about initiation of floral primordia, the latter condition being designated by C.

This interaction takes place in condition (1) above. In condition (2), the reac-

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Part of the equipment was obtained through a grant to Dr. K. C. Hamner from the American Association for the Advancement of Science.



tions leading to floral initiation do not take place because of limitation in the effectiveness of B. Under condition (3), floral initiation does not take place because of limitation in the effectiveness of A. Under condition (4), the reactions are limited by the ineffectiveness of both A and B. In this last case exposure to very short cycles of alternating light and darkness results in decreased effectiveness of both A and B, regardless of the previous treatments; subsequent to such cycles, conditions must be attained which will result in an increase in the effectiveness of both A and B in order that floral initiation may result.

Thus the cocklebur plants growing on a long photoperiod in the greenhouse remain vegetative under condition (2). Such plants may reach a high degree of effectiveness of A, and are induced to flower by a single treatment of a long dark period, since their flowering is limited only by the effectiveness of B. If such plants are removed from the greenhouse and treated with a number of short cycles of alternating light and darkness, however, the effectiveness of A decreases. Subsequently such plants must be exposed to a photoinductive cycle, which includes both a relatively long light period and a relatively long dark period.

The present work deals with these various interrelationships and the influence of temperature, light intensity and duration, and other environmental factors upon these interactions.

General methods

Two short-day plants, cocklebur and Biloxi soybean, were employed. The experiments with cocklebur were performed during the summer and early autumn of 1939, while the experiments with soybean were conducted during the spring and summer of 1940.

The cockleburs were grown from seed collected in the vicinity of Chicago in November, 1938. The burs were stored out-of-doors until desired for use. The seeds were removed from the fruit, scratched slightly to rupture the seed coats, and planted within a few days in light potting soil. Before germination of the seeds, the flats were placed on a well-lighted bench in the greenhouse, where supplementary illumination of about 100 foot-candles at the soil surface was provided by Mazda filament light from sundown until 2:00 A.M., making a photoperiod never less than 18 hours. The conditions on this bench will subsequently be referred to as those of long photoperiod. Cocklebur and other short-day plants have been grown under these conditions for more than a year without showing evidence of floral initiation when dissected. When the seedlings were about 4 inches tall they were transplanted to $3\frac{1}{2}$ -inch clay pots.

Four seeds of Biloxi soybean were planted in $4\frac{1}{2}$ -inch clay pots containing garden soil and placed immediately on long photoperiod. After the seedlings had emerged and the cotyledons were well expanded, two were removed from each pot, care being taken to secure maximum uniformity of plants.

The plants were watered twice daily with tapwater and were frequently given a double-strength modified SHIVE's (12) solution. They were exposed to conditions of long photoperiod until treated. The cockleburs were 6-8 weeks old and the soybeans 4-5 weeks old when the experiments were started. At the end of a specific treatment, if the intensity of sunlight in the greenhouse was 1000 foot-candles or more, the plants were placed immediately on long photoperiod; if less than this, the cockleburs were placed in a room at 70° F. illuminated with a carbon arc and the soybeans under white fluorescent light (1000 foot-candles or more) until the sunlight was sufficiently strong for them to be placed under conditions of long photoperiod. After treatment, all plants remained under conditions of long photoperiod for 3 weeks, at which time dissections were made. At the start of each experiment, and from time to time during them, plants which had been continuously under conditions of long photoperiod were dissected to make certain that no floral initiation had taken place. Illumination was supplied by sunlight, Mazda filament light, carbon arc light, or white fluorescent light. Intensities were determined in foot-candles by means of a standardized Weston photometer.

In experiments 1 and 2 with cocklebur, and in experiments 8-10 with soybean, the plants were treated with short cycles of alternating light and darkness, each consisting of 3 hours of darkness followed by 3 minutes of light. In the remaining experiments with cocklebur (experiments 3-7) these short cycles consisted of 2 hours of darkness followed by 2 minutes of light. The light intensity of these short photoperiods was 200 foot-candles unless otherwise indicated.

Procedure and results

Cocklebur

The experiments were of two general types: one dealt with the effectiveness of short alternating periods of darkness and light under various conditions in preventing floral initiation, and the other with the effects of light and temperature—both separate and in combination—during the photoperiod on floral initiation and development.

A. EFFECTIVENESS OF SHORT ALTERNATING PERIODS OF DARKNESS AND LIGHT UNDER VARIOUS CONDITIONS IN PREVENTING FLORAL INITIATION

It has been shown that vegetative cockleburs kept constantly on long photoperiod may fail to be induced if just subsequent to a long photoperiod and prior to the 15-hour dark period a series of relatively short cycles of alternating light and dark periods are given (6). The same procedure was employed here in the first of these experiments, but in all subsequent ones it was slightly modified.

EXPERIMENT 1.—The first experiment was begun June 29, 1939. One hundred and sixty plants growing on long photoperiod were selected for uniformity. Ten of

these, designated continuous long photoperiod controls, were left on conditions of long photoperiod. Twenty were placed in a darkroom immediately; of these, ten were given a 15-hour and ten a 36-hour continuous dark period and then returned to conditions of long photoperiod. One group of ten plants was given a single short cycle of 3 hours of darkness and 3 minutes of light, subjected to a 15-hour dark period, and then returned to conditions of long photoperiod; another lot was given two of these short cycles, followed by a 15-hour dark period, and returned to long photoperiod; and so on. The maximum number of short cycles which any lot received was twelve. The remaining ten plants were given twelve of these cycles and returned immediately to long photoperiod without being exposed to a 15-hour dark period. The results (table 1) indicate that the cyclic treatment,

TABLE 1

EFFECT OF NUMBER OF SHORT CYCLES, EACH CONSISTING OF 3 HOURS OF DARKNESS AND 3 MINUTES OF LIGHT, ON FLORAL INITIATION IN COCKLEBUR WHEN PLANTS RECEIVED A LONG DARK PERIOD IMMEDIATELY SUBSEQUENT TO TREATMENT WITH SHORT CYCLES. TEN PLANTS IN EACH LOT

No. of short cycles. . . .	0	0	0	1	2	3	4-12
Length of dark period following short cycles (hours)	0	15	36	15	15	15	15
Flowering condition after 14 days on long photoperiod.	10 veg.	10 fl. pr.	10 fl. pr.	4 fl. pr., 6 veg.	10 veg.	10 veg.	10 veg.

following a long photoperiod, may prevent the effectiveness of a subsequent 15-hour dark period in initiating floral primordia.

EXPERIMENT 2.—On the basis of the results of experiment 1, a second was carried out in August, 1939. Three relatively short cycles of 3-hour dark periods and 3-minute light periods, shown by the preceding results to be sufficient to prevent initiation, were used. Upon dissection, however, it was found that many of the controls (which received a 15-hour dark period following the three cycles) had initiated floral primordia.

June 29 was intermittently cloudy, and the light intensity was therefore rather low. Experiment 1 was begun at 10:00 A.M. on that day, so these plants had been subjected to approximately 5 hours of low-intensity light. Experiment 2 was started at 5:00 P.M. following an exceptionally bright day. Since this difference might have had an important effect on the number of relatively short alternating light and dark periods required to inhibit floral initiation, a thorough investigation was made of the effects of the environmental factors on the effectiveness of the cyclic treatment.

For uniformity, all remaining experiments (3-10) were begun at 5:00 P.M. fol-

lowing at least 10 hours of sunlight of 3000-5000 foot-candles' intensity. Experiments 3-7 were carried out during the first week of September, and dissections were made during the first week of October.

EXPERIMENT 3.—Three groups of vegetative plants, each consisting of 168 plants, were exposed continuously to light for 36 hours, one group at an intensity of 50 foot-candles, another at 500, and another at 5000. From each of these groups sixteen plants were placed in the darkroom. Of each group of sixteen, half received a 12-hour and half a 36-hour continuous dark period and then returned to long photoperiod. From each of the original groups, 152 plants were given short cycles of 2 hours of darkness followed by 2 minutes of light of 200 foot-

TABLE 2

EFFECT ON FLORAL INITIATION IN COCKLEBUR OF VARIOUS LIGHT INTENSITIES DURING 36-HOUR PHOTOPERIOD BEFORE CYCLIC TREATMENT CONSISTING OF 2 HOURS OF DARKNESS AND 2 MINUTES OF LIGHT. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF EIGHT PLANTS

No. of short cycles.....	0	0	0	1	2	3	4	5-18	18
Length of dark period following short cycles (hours)...	0	12	36	12	12	12	12	12	0
Intensity of 36-hour photo-period:									
50 f.c.....	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
500 f.c.....	8 veg.	8 fl.	8 fl.	8 fl.	5 fl., 3 veg.	8 veg.	8 veg.	8 veg.	8 veg.
5000 f.c.....	8 veg.	8 fl.	8 fl.	8 fl.	8 fl.	6 fl., 2 veg.	4 fl., 4 veg.	8 veg.	8 veg.

candles at 70° F. Lots of eight plants were then removed from each group at the end of every cycle and subjected to a 12-hour continuous dark period before being returned to long photoperiod. One set of plants, however, which had received each of the light intensities prior to the cyclic treatment, was returned directly to long photoperiod after eighteen cycles, no 12-hour dark period having preceded this return. Eight plants, selected for controls for experiments 3-7 and left on long photoperiod, were vegetative at the conclusion of the experiment.

The results (table 2) indicate the reason for the variation observed between experiments 1 and 2. Apparently the degree of effectiveness of the cyclic treatment is directly related to the intensity and duration of light which the plants receive just prior to the short cycles of darkness and light; that is, plants exposed to high-light intensity preceding the cyclic treatment require more of these short cycles to prevent subsequent floral initiation than do those exposed to a relatively low-light intensity prior to the cyclic treatment.

Since these short cycles were effective in inhibiting subsequent floral initiation in the treatments, the effect of continuous photoperiod of low light intensity, as compared with these short cycles, was next determined. An attempt was made also to determine the effect of variation in the light intensity during the short photoperiods of these short cycles.

EXPERIMENT 4.—Four hundred and eighty-eight cocklebur plants, growing on long photoperiod, were selected for uniformity. Sixteen of these were placed in the darkroom, eight being returned to long photoperiod after a 12-hour and

TABLE 3

EFFECT ON FLORAL INITIATION IN COCKLEBUR OF CYCLIC TREATMENT CONSISTING OF 2 HOURS OF DARKNESS AND 2 MINUTES OF LIGHT OF VARIOUS INTENSITIES AND OF CONTINUOUS LIGHT OF LOW INTENSITY. EACH LOT OF EIGHT PLANTS

No. of cycles.....	1	2	3	4	5	6	7	8	9-18	18
No. of hours of continuous darkness following cyclic treatment.....	12	12	12	12	12	12	12	12	12	0
Flowering condition after 21 days on long photoperiod, when cycle consisted of:										
2 hours of darkness plus 2 minutes of light at:										
2000 f.c.....	8 fl.	8 fl.	5 veg., 3 fl.	4 veg., 4 fl.	7 veg., 1 fl.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
200 f.c.....	8 fl.	8 fl.	2 veg., 6 fl.	4 veg., 4 fl.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
20 f.c.....	8 fl.	8 fl.	3 veg., 5 fl.	5 veg., 3 fl.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
2 hours of continuous light at 20 f.c.	8 fl.	8 fl.	8 fl.	8 fl.	8 fl.	8 fl.	8 fl.	7 veg., 1 fl.	8 veg.	8 veg.

eight after a 36-hour continuous dark period. These control plants initiated primordia. One hundred and twelve plants were placed under continuous light of 20 foot-candles. Every 2 hours for the first 20 and every 4 hours for the next 16, lots of eight plants each were removed to the darkroom for 12 hours and then immediately returned to long photoperiod. Three lots of 120 plants each were given short cycles with the 2-minute light periods at intensities of 20, 200, and 2000 foot-candles, respectively. Each of the eighteen short cycles consisted of 2 hours of darkness followed by 2 minutes of light. Lots of eight plants were removed from each intensity at the end of every cycle, given a 12-hour continuous dark period, and returned to long photoperiod. At the completion of eighteen cycles an additional group of eight plants from each intensity was returned directly to long photoperiod with no intervening dark period.

The results (table 3) show that for cocklebur, cycles with brief periods of light of all three intensities are effective in inhibiting floral initiation, even though the plants are given a subsequent 12-hour dark period. Those plants given cycles with 2-minute light periods at 2000 foot-candles required seven cycles for such inhibition, while those given 20 or 200 foot-candles required only five cycles. The plants given 18 hours or more of continuous light of low intensity (20 foot-candles) also failed to initiate floral primordia, even when subsequently given a 12-hour dark period.

Table 3 indicates that light intensity during the treatment of short cycles may influence the results. In the following experiment an attempt was made to determine what effect temperature might have on treatment with short cycles.

TABLE 4

EFFECT ON FLORAL INITIATION IN COCKLEBUR OF VARIOUS TEMPERATURES DURING SHORT CYCLES CONSISTING OF 2-HOUR DARK AND 2-MINUTE LIGHT PERIODS. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF EIGHT PLANTS

No. of short cycles. . .	1	2	3	4	5	6-18	18
Length of dark period following short cycles (hours)	12	12	12	12	12	12	0
Flowering condition after 21 days on long photoperiod, following short cycles at:							
40° F.	8 fl.	8 fl.	1 veg., 7 fl.	2 veg., 6 fl.	7 veg., 1 fl.	8 veg.	8 veg.
75° F.	8 fl.	8 fl.	2 veg., 6 fl.	4 veg., 4 fl.	8 veg.	8 veg.	8 veg.
95° F.	8 fl.	8 fl.	1 veg., 7 fl.	3 veg., 5 fl.	8 veg.	8 veg.	8 veg.

EXPERIMENT 5.—The continuous long photoperiod controls of this experiment are those of experiment 3, and the 12- and 36-hour continuous dark-period controls (not given the short-cycle treatment) are those of experiment 4. Three hundred and sixty plants were divided into three lots of 120 plants each and subjected to the short-cycle treatment at 40°, 75°, and 95° F., respectively. Lots of eight plants were removed from each group at the end of every short cycle, given a 12-hour continuous dark period at 70° F., and returned to long photoperiod. The results of this experiment are given in table 4. The differences in response to the three temperature groups are insignificant and indicated that the temperatures used were without influence on the effectiveness of the alternating dark and light periods in preventing floral initiation.

B. DURATION AND INTENSITY OF LIGHT AND TEMPERATURE REQUIREMENTS OF PHOTOPERIOD PRECEDING DARK PERIOD

Since it has been observed that floral initiation could be prevented by treating plants with short cycles of alternating darkness and light before subjecting them

to a continuous dark period of 9 hours or more, it was decided to test the conditions under which plants might resume a condition permitting initiation by giving some which had received eighteen short cycles a controlled photoperiod before subjecting them to the continuous dark period of at least 12 hours.

EXPERIMENT 6.—Eighty vegetative cocklebur plants were selected from long photoperiod and given eighteen cycles of alternating 2-hour dark periods and 2-minute light periods (200 foot-candles) at 75° F. Ten plants were returned to long photoperiod immediately after the eighteen cycles, and served as checks. The remaining seventy were divided equally into seven groups, each of which was given one of the following treatments:

- 2 hours of 2000 f.c. carbon arc light + 22 hours darkness
- 4 hours of 2000 f.c. carbon arc light + 20 hours darkness
- 6 hours of 2000 f.c. carbon arc light + 18 hours darkness
- 8 hours of 2000 f.c. carbon arc light + 16 hours darkness
- 10 hours of 2000 f.c. carbon arc light + 14 hours darkness
- 10 hours of 100 f.c. Mazda filament light + 14 hours darkness
- 10 hours of 10 f.c. Mazda filament light + 14 hours darkness

Each cycle was repeated three times, at the conclusion of which the plants were returned immediately to long photoperiod, where they remained for 21 days.

TABLE 5
FLOWERING RESPONSE OF COCKLEBUR TO VARIOUS PHOTOINDUCTIVE CYCLES
GIVEN AFTER 18 CYCLES OF ALTERNATING 2-HOUR DARK AND 2-MINUTE
LIGHT PERIODS. EACH PHOTOINDUCTIVE CYCLE REPEATED THREE TIMES.
DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF
TEN PLANTS

PHOTOINDUCTIVE TREATMENT	NO. OF PLANTS	
	VEG.	FL. PR.
2 hours of 2000 f.c.+22 hours of darkness.....	2	8
4 hours of 2000 f.c.+20 hours of darkness.....	0	10
6 hours of 2000 f.c.+18 hours of darkness.....	0	10
8 hours of 2000 f.c.+16 hours of darkness.....	0	10
10 hours of 2000 f.c.+14 hours of darkness.....	0	10
10 hours of 100 f.c.+14 hours of darkness.....	0	10
10 hours of 10 f.c.+14 hours of darkness.....	10	0
Controls (continuously on long day).....	10	0

In two groups, the controls and those given three cycles of 10 foot-candles Mazda filament light for 10 hours, there was no initiation of floral primordia (table 5). Two of the plants exposed to three cycles of 2000 foot-candles of carbon arc light for 2 hours and 22 hours of darkness also failed to initiate primordia. The apical meristems had become flower primordia in all other plants. The stages of

that the longer the duration and the stronger the intensity of the photoperiod, the more rapid the subsequent rate of floral development.

EXPERIMENT 7.—One group of workers (4, 5, 10, 11, 13, 14) has reported that variations in temperature during the photoperiod may alter the length of the light period required for floral initiation in many plants. Others (4, 5, 7, 9, 10) have shown that the length or number of critical dark periods may be adversely affected by temperature conditions during the dark period.

Experiment 7 shows that the temperature conditions prevailing during the photoperiod affect initiation and subsequent flower development. It varies from ex-

TABLE 6

FLOWERING RESPONSE OF COCKLEBUR TO VARIATIONS IN TEMPERATURE DURING PHOTOPERIOD OF VARIOUS PHOTOINDUCTIVE CYCLES FOLLOWING 18 CYCLES OF ALTERNATING 2-HOUR DARK AND 2-MINUTE LIGHT PERIODS. TEMPERATURE OF DARK PORTION OF PHOTOINDUCTIVE CYCLE 70° F. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF EIGHT PLANTS

	TEMPERATURE DURING PHOTOPERIOD					
	40° F.		75° F.		95° F.	
	VEG.	FL.	VEG.	FL.	VEG.	FL.
After 18 cycles of alternating light and dark periods, subjected to three photoinductive cycles:						
2 hours of 2000 f.c.+14 hours dark.	7	1	1	7	7	1
4 hours of 2000 f.c.+14 hours dark.	1	7	0	8	0	8
6-10 hours of 2000 f.c.+14 hours dark.	0	8	0	8	0	8
Controls (continuously on long photoperiod).	8 vegetative, 0 flowering					
After 18 cycles transferred directly to long photoperiod.	8 vegetative, 0 flowering					
After 18 cycles transferred to 12-hour dark period, then to long photoperiod.	8 vegetative, 0 flowering					
Transferred to 14-hour dark period immediately after photoperiod, then to long photoperiod.	0 vegetative, 8 flowering					

periment 6 in two respects: (a) all plants were given 12-hour dark periods, and (b) three temperature conditions prevailed during the photoperiod. The results (table 6) show that initiation was inhibited at low and high temperatures, but only when the treatment consisted of three cycles of 2 hours of 2000 foot-candles of carbon arc light and 12-hour dark periods. Significant variations were found in the rate of development of the flower primordia (fig. 2). In general, at the higher temperatures there was greater increase in the rate of flower-bud development than at the low temperature. At all three temperatures a marked increase in the rate of flower-bud development followed three photoinductive cycles whose photoperiods

were 8 hours long. Plants given three cycles with 10-hour photoperiods had not developed so far as those given an 8-hour photoperiod.

Biloxi soybean

Relatively short alternating dark and light periods were inserted into the photoinductive cycle of another short-day plant to test the effectiveness of the cyclic treatment in preventing floral initiation. Soybean was selected because of the data available relative to its photoperiodic relationships. As in the case of cocklebur, short cycles were given between the photoperiod and the dark period of each photoinductive cycle, and similar short cycles were inserted between each photoinductive cycle (following the dark period of each photoinductive cycle).

EXPERIMENT 8.—On May 3, 1940, 250 soybeans which had been on long photoperiods for 5 weeks were selected. Five lots of ten plants each were used as controls and given one of the following treatments:

1. Left continuously on long photoperiod.
2. Given 240 hours of white fluorescent light of 600 foot-candles, then returned to long photoperiod.
3. Given 240 hours of continuous darkness, then returned to long photoperiod.
4. Given 80 short cycles (240 hours) consisting of 3 hours of darkness and 3 minutes of light (200 foot-candles Mazda filament light), then returned to long photoperiod.
5. Given ten photoinductive cycles consisting of 10 hours of light followed by 14 hours of darkness, then returned to long photoperiod.

All plants of lots 1-4 were vegetative, but those of lot 5 were flowering at the conclusion of the experiment. The remaining 200 plants were divided into two equal lots, and at 5:00 P.M., following a photoperiod of more than 10 hours of sunlight of 3000-5000 foot-candles, one (group A) was placed in the darkroom, while the other (group B) was placed in a darkroom equipped to give frequent short periods of Mazda filament light.

Following a 14-hour continuous dark period, the 100 plants of group A were removed to the short-cycle room. One lot of ten plants received one short cycle consisting of 3 hours of darkness followed by 3 minutes of light, then placed under white fluorescent light (600 foot candles) for 10 hours, and finally returned to the darkroom for 14 hours. Another lot received two of these short cycles following the 14-hour dark period; a third received three of these cycles, etc. Each of these treatments (10 hours of light plus 14 hours of darkness plus 1-10 short cycles) was repeated ten times. At the conclusion of a given treatment the plants were returned to conditions of long photoperiod in the greenhouse—provided the intensity of the sunlight exceeded 1000 foot-candles—or were placed under white fluorescent light until such time as the intensity of the sunlight was 1000 foot-candles or more.

Further, any group which completed the treatment during the late afternoon was kept under the fluorescent light until the following morning.

Of the 100 plants of group B, placed in the short-cycle room at the start of the experiment, one lot of ten plants remained for one short cycle, another for two, and so on. Upon removal from this room, the plants were given a 14-hour continuous dark period, followed by 10 hours of white fluorescent light, and then returned to the short-cycle room for the specified number of short cycles. Each of these treatments was likewise repeated ten times, and at the conclusion of a given treatment the plants were returned to conditions of long photoperiod.

The results (fig. 3) are striking, in that short cycles inserted before the dark period were ineffective in preventing floral initiation, while the inhibitory effect of short cycles following the dark period appears rhythmical. Although all plants were flowering when the short cycles preceded the dark period, some inhibitory effect on flowering is evidenced by the average number of nodes with flowers per plant. Figure 4 shows that the same general trends of inhibition of the number of nodes with flowers is affected by short cycles inserted either before or after the dark period, as well as by the effect of short cycles inserted after the dark period on the total number of plants flowering. A comparison of the average number of nodes per plant with flower primordia of comparable treatments from groups A and B shows that in no instance was there a greater number of nodes with primordia when the short cycles followed the dark period than when they preceded it. Evidently short cycles inserted after the dark period of the photoinductive cycle are much more effective in suppressing the flowering response.

EXPERIMENT 9.—This experiment was performed at the same time as experiment 8, hence the controls for both were the same. Seven hundred soybeans which had been on continuous long photoperiods were selected for uniformity and divided into seven groups of 100 plants each. These groups were subjected to cycles as follows:

1. 10 hours of light, then 14 hours of continuous darkness.
2. 10 hours of light followed by one short cycle (3 hours of darkness and 3 minutes of 200 foot-candles of Mazda filament light), then 14 hours of continuous darkness.
3. 10 hours of light followed by five short cycles, then 14 hours of continuous darkness.
4. 10 hours of light followed by ten short cycles, then 14 hours of continuous darkness.
5. 10 hours of light followed by 14 hours of continuous darkness, then one short cycle.
6. 10 hours of light followed by 14 hours of continuous darkness, then five short cycles.

7. 10 hours of light followed by 14 hours of continuous darkness, then ten short cycles.

Ten plants from each group were removed to conditions of long photoperiod at the end of each cycle, ten cycles being required to remove all plants from any group.

The effect of the short-cycle treatments, combined with a varying number of photoinductive cycles, upon the total number of plants flowering per treatment is given in table 7. In no instance were flowers induced when less than three photoinductive cycles were used; and as has been previously reported (1, 3, 9), maximum flowering occurred when a relatively large number of photoinductive cycles were given.

TABLE 7

EFFECT ON FLORAL INITIATION IN SOYBEAN OF VARYING NUMBER OF PHOTOINDUCTIVE CYCLES WHICH INCLUDE SHORT CYCLES CONSISTING OF 3 HOURS OF DARKNESS AND 3 MINUTES OF LIGHT (200 F.C.). DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF TEN PLANTS

TREATMENT	No. OF PLANTS FLOWERING AFTER NUMBER OF PHOTOINDUCTIVE CYCLES AS INDICATED									
	1	2	3	4	5	6	7	8	9	10
No. of short cycles inserted in photoinductive cycle before 14-hour dark period:										
1	0	0	2	2	8	8	9	10	10	10
5	0	0	0	0	0	3	0	3	8*	9*
10	0	0	0	0	0	2	1	6	8	10
No. of short cycles inserted in photoinductive cycle after 14-hour dark period:										
1	0	0	0	1	4	9	10	10	10	10
5	0	0	0	0	0	0	0	0	2	1
10	0	0	0	0	2	0	1	5	3	2
No short cycles included in photoinductive cycle	0	0	6	8	10	10	10	10	10	10
Continuous long photoperiod	0 flowering; 10 vegetative									

* One dead plant in this lot.

One short cycle inserted either before or after the dark period of the photoinductive cycle had some effect when relatively few photoinductive cycles were given (seven or less), but when eight or more comprised the treatment all plants showed some degree of flowering. If five short cycles were so inserted before or after one to eight photoinductive cycles, floral expression was much more inhibited. Five short cycles inserted before the dark period of nine or ten photoinductive cycles did not materially inhibit initiation, but when inserted after the dark period, very few plants were flowering. In general, when ten short cycles were included the floral response ranged between these two.

EXPERIMENT 10.—This was designed to test the results obtained in experiment 8 as well as to compare the effectiveness of continuous light of low intensity with short cycles which included 3-minute light periods of low and high intensity in preventing floral initiation in soybean.

The plants were 30 days old at the start of the various treatments and had been under long photoperiod in the greenhouse for that entire time. The same precautions employed before and after treatments in other experiments were used here. Table 8 gives the relative humidity and temperature readings made every 3 hours, and maximum, minimum, and averages for the five rooms used. Fluctuations in both temperature and relative humidity were gradual.

White fluorescent light (1200 foot-candles) was used for the 10-hour photoperiods, and similar light of 20 foot-candles was used for the low intensity con-

TABLE 8

Room	TEMPERATURE (° F.)			PERCENTAGE RELATIVE HUMIDITY		
	MAX.	MIN.	AVE.	MAX.	MIN.	AVE.
Darkroom.....	86	76	82	80	63	77
Continuous light (20 f.c.)....	88	79	84	80	47	75
Continuous light (1200 f.c.)...	89	79	85	85	53	75
Short cycle (200 f.c.).....	84	78	81	84	64	75
Short cycle (20 f.c.).....	83	76	80	87	64	75

tinuous light treatment. The 3-minute light periods of the cyclic treatment were of 20 and 200 foot-candles Mazda filament light.

On July 8, 1940, 800 soybeans were selected for uniformity. Twenty of these were dissected immediately and every node was found to be vegetative. Eighty more were designated controls and divided into four lots of twenty plants, each receiving one of the following treatments:

1. Continuous long photoperiod. All plants vegetative.
2. Ten photoinductive cycles consisting of 10-hour photoperiods and 14-hour dark periods, then returned to long photoperiod. All plants flowering at conclusion of experiment. Average number of nodes with flowers, 5.3.
3. Ten days of continuous light of 20 foot-candles, then returned to long photoperiod. All plants vegetative at conclusion of experiment.
4. Ten days of continuous light of 1200 foot-candles, then returned to long photoperiod. All plants vegetative at conclusion of experiment.

Two groups of 200 plants each were given ten cycles consisting of 10 hours of light, followed by 14 hours of continuous darkness, followed by a given number of short cycles of alternating 3-hour dark periods and 3-minute light periods. One group received a high-intensity light (200 foot-candles) during the 3-minute period,

while the other group received a low-intensity light (20 foot-candles) during this period. One lot of twenty plants from each group received 1, 4, 6, 8, 10, 11, 12, 13, 14, and 15 short cycles. The remaining plants were divided into fifteen lots of twenty plants, each lot receiving ten cycles of 10 hours of bright light (1200 foot-candles) plus 14 hours of continuous darkness plus a given number of hours of continuous light at 20 foot-candles. One lot received 3 hours of the low-intensity light

TABLE 9

EFFECT ON FLORAL INITIATION IN SOYBEAN OF SHORT CYCLES OF 3 HOURS OF DARKNESS AND 3 MINUTES OF LIGHT OF VARIOUS INTENSITIES OR CONTINUOUS LIGHT OF LOW INTENSITY FOLLOWING EACH DARK PERIOD OF TEN PHOTOINDUCTIVE CYCLES. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF TWENTY PLANTS

NO. OF SHORT CYCLES FOLLOWING EACH DARK PERIOD OF PHOTOINDUCTIVE CYCLE	INTENSITY OF LIGHT OF SHORT CYCLES								CONTINUOUS LIGHT (20 F.C.)*			
	20 F.C.				200 F.C.							
	NO. OF PLANTS			AVE. NO. NODES WITH FL.	NO. OF PLANTS			AVE. NO. NODES WITH FL.	NO. OF PLANTS			AVE. NO. NODES WITH FL.
	FL.	VEG.	DEAD		FL.	VEG.	DEAD		FL.	VEG.	DEAD	
1.....	20	0	0	4.2	19	1	0	4.1	20	0	0	3.5
2.....									2	17	1	2.5
3.....									0	20	0	0.0
4.....	5	14	1	2.0	0	20	0	0.0	0	19	1	0.0
5.....									1	19	0	2.0
6.....	6	11	3	2.5	4	14	2	1.8	9	10	1	1.7
7.....									5	13	2	2.0
8.....	9	4	7	4.0	15	2	3	3.5	3	12	5	2.7
9.....									0	14	6	0.0
10.....	1	6	13	2.0	1	11	8	2.0	0	13	7	0.0
11.....	0	5	15	0.0	1	9	10	2.0	0	11	9	0.0
12.....	0	3	17	0.0	0	0	20	0.0	0	6	14	0.0
13.....	0	2	18	0.0	1	3	16	3.0	0	4	16	0.0
14.....	3	3	14	4.0	0	2	18	0.0	0	12	8	0.0
15.....	1	2	17	3.0	0	0	20	0.0	0	6	14	0.0

* Each short cycle represents 3 hours of continuous light.

following the dark period, a second received 6 hours, a third 9 hours, etc., to a maximum of 45 hours.

A number of the plants of some treatments did not survive the experimental period. Apparently this was due to the relatively short length and low intensity of the 10-hour photoperiods which made up each photoinductive cycle. The results of experiment 10 are shown in table 9.

The results of experiment 10 correspond in general to those obtained in experiment 8 (figs. 3, 5). Relatively short alternating periods of light and darkness, as well as continuous light of low intensity, when inserted after the 14-hour dark

period of the photoinductive cycle may reduce the photoinductive effect of such a cycle. The effectiveness of such treatment appears rhythmical (fig. 5). There is a rapid decrease in the number of plants flowering when 2-4 short cycles were so in-

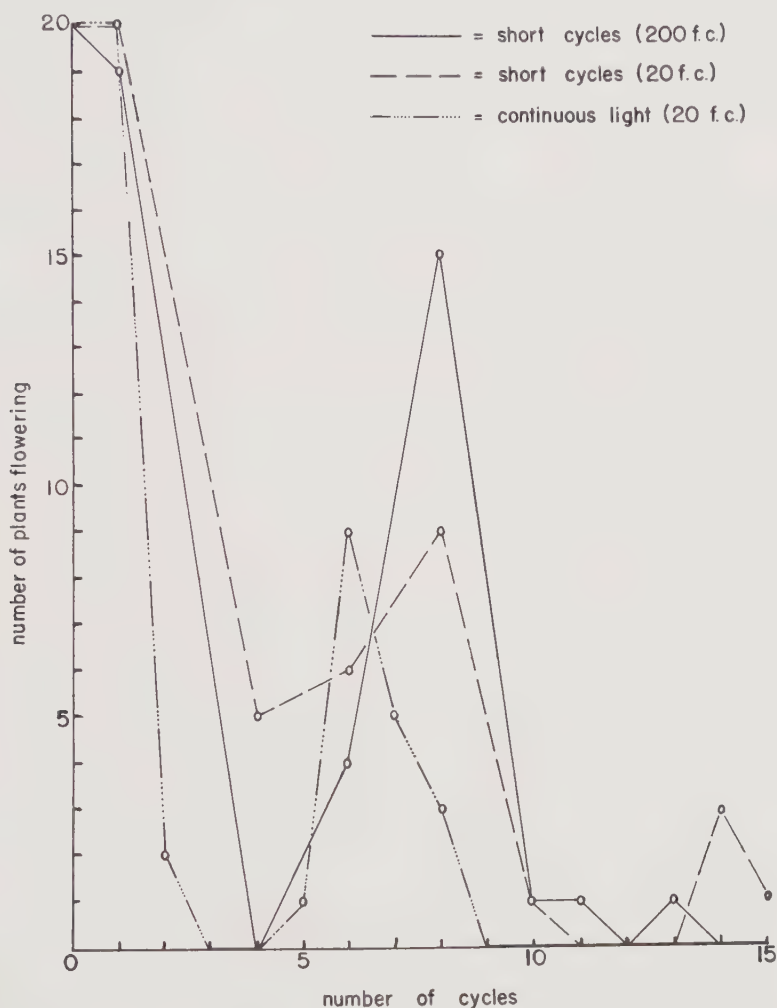


FIG. 5.—Effect on initiation in Biloxi soybean of ten photoinductive cycles which include short cycles (3 hours of darkness and 3 minutes of light or 3 hours of continuous light of low intensity) inserted after 14-hour dark period. Each short cycle represents lapse of 3 hours. Data taken 21 days after completion of treatment.

serted, then a rapid increase in the number flowering (5-9 short cycles), which is followed by another decrease. An insufficient number of the plants receiving 10-15 short cycles survived to warrant a definite statement as to their effect;

however, there is indication that an increase in the number flowering would have occurred. The average number of nodes per plant with flowers and the total number flowering of the various treatments roughly follows the same type curve (figs. 3-5). Whenever short cycles were introduced into the photoinductive cycle, regardless of their number or their place of insertion, fewer nodes per plant had flowers than occurred on plants subjected to a corresponding number of photoinductive cycles but without the short cycles.

Discussion

These results substantiate previous findings (1, 3, 6, 7, 9) that in both cocklebur and Biloxi soybean photoperiodic induction takes place as a result of interaction between conditions brought about during exposure both to light and to darkness. This cyclic alternation of light and dark periods, when of appropriate duration, intensity, and frequency, results in photoperiodic induction. Under certain of these conditions cocklebur requires one such photoinductive cycle, and Biloxi soybean three, for photoperiodic induction. The necessary duration of the light and the dark period is dependent upon a number of factors, such as intensity and quality of light, temperature, age of plants, age and position of leaves, and so on (1-10). At present the nature of the interactions which result in photoperiodic induction are unknown.

The results of the present work indicate that cocklebur, when exposed to relatively bright light, may undergo certain changes or reactions which so alter the plant that if it is immediately exposed to a relatively long dark period it becomes photoperiodically induced and may subsequently initiate floral primordia. If a plant which has been exposed to such a period of bright light is exposed to relatively short alternating periods of light and darkness, however, the effectiveness of the photoperiod disappears, and even though subsequently subjected to a long dark period, initiation does not occur. The same results brought about by these relatively short alternating light and dark periods may also be produced by exposing a plant to continuous light of very low intensity, although in the latter instance the time required for the apparent lowering of the effectiveness of the previous photoperiod is much longer.

During photoperiods of bright light the establishment of A may occur at a rapid rate and there may result a higher degree of its effectiveness at the end of the photoperiod than at the beginning. Similarly, during photoperiods with low-intensity light the rate of establishment of A may be lower, and in complete darkness or during photoperiods with a very low light intensity its rate of establishment may actually be lower than the rate of its destruction, resulting in a lower level of effectiveness at the end of the period than at the beginning. If such were the case, it would be expected that a low temperature during treatment with

short cycles would result in a lower rate of decrease of the conditions brought about during the photoperiod. Such does not seem to be the case, since variations in temperature produced only slight differences in the number of short alternating cycles of light and dark periods necessary to prevent initiation.

Whatever the reasons, it seems apparent that conditions prevailing during the photoperiod influence the relative effectiveness of A. This is indicated by the preceding results, which show that a greater number of short cycles must be used to prevent photoperiodic induction following a period of bright light than following a period of relatively low light intensity; and that following a series of eighteen alternating cycles of short light and dark periods the length and intensity of photoperiods determine the subsequent return to a condition resulting in photoperiodic induction. It would appear also that the number of short cycles needed to prevent photoperiodic induction might serve as a measure of the accumulated effectiveness of A during previous exposure to light. Utilization of this method may give a quantitative basis for the measurement of the rate and degree of reaction on the part of the plant to exposure to photoperiods at various light intensities, temperatures, etc. Such a procedure has been carried out, and the results indicate that whatever the conditions brought about during exposure to the photoperiod, they take place to a greater degree during photoperiods of high light intensity than during those of lower intensity. A low temperature during the photoperiod apparently results in a slower rate of establishment of the effectiveness of A. These results are in general agreement with the response of Biloxi soybean to experiments of a similar nature and indicate the similarity in response between the two plants.

These results with cocklebur and Biloxi soybean may lead to a possible interpretation of apparently conflicting results obtained by other workers. It has been noted from time to time that photoperiodically sensitive plants may respond differently, depending upon the time of day that they receive supplementary illumination. Thus it has been the general practice, when it was desirable to expose plants to long photoperiodic conditions, to expose them to natural daylight and supplement this by continued exposure to light from Mazda lamps into the night. Contrary to this general practice, the supplementary illumination has occasionally been given from a designated time during the night until sunrise. In one case supplementary illumination followed the photoperiod of high light intensity and in the other case preceded it. A possible explanation of the conflicting results may be that with certain plants the photoperiodic induction more readily results if a period of bright illumination immediately precedes exposure to darkness. In this connection, the experiments with Biloxi soybean are of interest. While the short cycles were partially effective in inhibiting initiation, whether they immediately preceded or immediately followed the dark period, there was considerable difference in their relative effectiveness—not only with reference to the number of

plants flowering but also with reference to the number of nodes per plant which possessed floral primordia. More evidence is needed before definite conclusions can be reached, but preliminary results indicate that treatment immediately following the dark period would prove most effective.

The indications which were obtained as to the cyclic changes in sensitivity on the part of the soybean are difficult to interpret, but it would seem that utilization of cycles other than those based on 24 hours may bring about ambiguous results because of cyclic changes in sensitivity on the part of the plant which seem to follow a 24-hour cycle. It may be that future work should involve treatment of the plants from the time they are planted until the end of the experiment to avoid such conditions.

The results of experiments with both cocklebur and Biloxi soybean at least indicate that a practical method of maintaining short-day plants in the vegetative condition would be to expose them to brief periods of illumination during the night. This should prove even more effective in maintaining the plants in a vegetative condition than the common practice of exposing them to continuous illumination of low light intensity during a part of the night.

Summary

1. In cocklebur, the conditions brought about during the photoperiod may be diminished to such a point that plants fail to initiate floral primordia when subsequently subjected to a 12-hour dark period. The methods employed are the insertion between the photoperiod and the dark period of the photoinductive cycle of a given number of short alternating periods of darkness and light or of a given number of hours of continuous light of low intensity. When Biloxi soybean was treated in the same manner there was no reduction in the number of plants flowering, although there was a decrease in the number of nodes with flowers.

2. In Biloxi soybean, short cycles of light and darkness, or continuous light of low intensity, when inserted following the dark period of each photoinductive cycle, reduced considerably the number of plants flowering as well as the average number of nodes per plant with flowers. This response appears rhythmical, and there are indications that utilization of cycles other than those under which the plant has been growing may bring about ambiguous results.

3. Effective short cycles consisted of either 3 hours of darkness and 3 minutes of light or 2 hours of darkness and 2 minutes of light.

4. The use of short alternating periods of darkness and light may be a more economical method of maintaining "short-day" plants in a vegetative condition than the common practice of exposing them to continuous supplementary illumination of low intensity.

5. The intensity of the light during these short periods apparently affects the response; and in cocklebur the temperature prevailing during the cyclic treatment had little, if any, effect.

6. A range of conditions imposed during the photoperiod of a photoinductive cycle can be directly correlated with the extent or degree of floral initiation shown by cocklebur subjected to the various treatments.

7. Following eighteen short cycles, cocklebur may regain the capacity of being induced and develop flowers if subjected to three photoinductive cycles consisting of photoperiods of 2 or more hours at 2000 foot-candles or 10 hours of 100 foot-candles and 14 hours of continuous darkness. Moderate to high temperatures during the photoperiod and an increase in the intensity and duration of the light favor establishment of the conditions brought about by the photoperiod as measured in these experiments.

This research was carried out under the guidance of Dr. K. C. HAMNER. The writer is grateful to him; and also to JOHN SKOK and AUBREY NAYLOR for their assistance with the experimental work.

DEPARTMENT OF BIOLOGY
LOUISIANA POLYTECHNIC INSTITUTE
RUSTON, LOUISIANA

LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Effectiveness of photoperiodic treatments of plants of different age. *BOT. GAZ.* 100:245-249. 1938.
2. ———, Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. *BOT. GAZ.* 101:806-817. 1940.
3. ———, Influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans. *BOT. GAZ.* 99:825-839. 1938.
4. ———, Photoperiodic perception in Biloxi soybeans. *BOT. GAZ.* 100:374-387. 1938.
5. ———, Photoperiodic responses of several varieties of soybeans. *BOT. GAZ.* 101:341-365. 1939.
6. HAMNER, K. C., Interrelation of light and darkness in photoperiodic induction. *BOT. GAZ.* 101:658-687. 1940.
7. HAMNER, K. C., and BONNER, JAMES, Photoperiodism in relation to hormones as factors in floral initiation and development. *BOT. GAZ.* 100:388-431. 1938.
8. HAMNER, K. C., and LONG, E. M., Localization of photoperiodic perception in *Helianthus tuberosus*. *BOT. GAZ.* 101:81-90. 1939.
9. LONG, E. M., Photoperiodic induction as influenced by temperature, relative humidity, and previous photoperiodic treatments. *BOT. GAZ.* 101:168-188. 1939.

10. PARKER, M. W., and BORTHWICK, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. *BOT. GAZ.* 101:145-167. 1939.
11. ROBERTS, R. H., The effect of temperature on the responses of plants to photoperiod. *Science* 85:290-291. 1937.
12. SHIVE, J. W., A three-salt nutrient solution for plants. *Amer. Jour. Bot.* 2:157-160. 1915.
13. STEINBURGER, R. A., and GARNER, W. W., Response of certain plants to length of day and temperature under controlled conditions. *Jour. Agr. Res.* 52:943-960. 1936.
14. THOMPSON, H. C., and KNOTT, J. E., The effect of temperature and photoperiod on the growth of lettuce. *Proc. Amer. Soc. Hort. Sci.* 30:507-509. 1933.

